

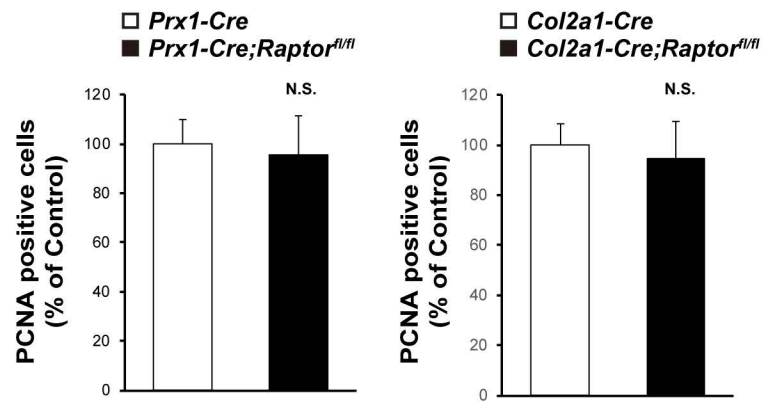
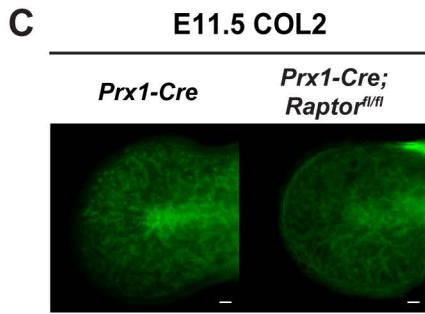
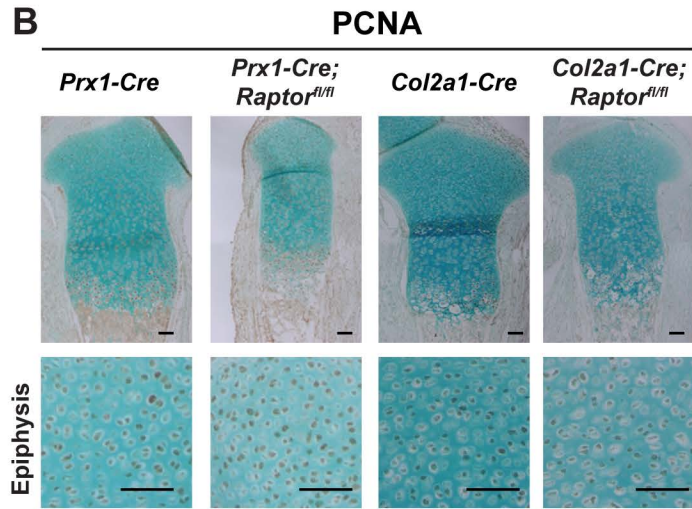
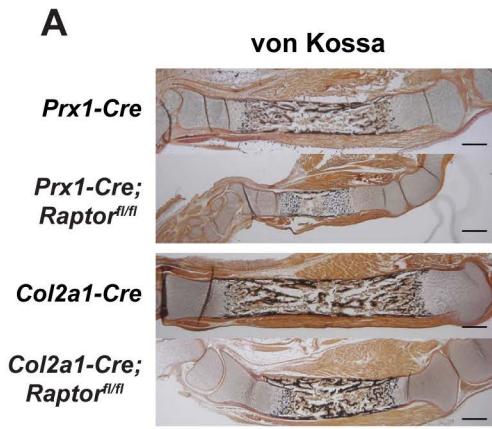
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**Supplemental Information**

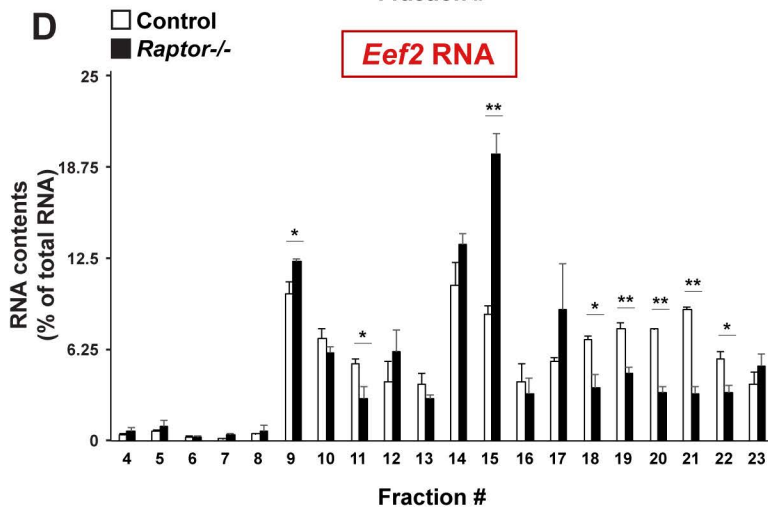
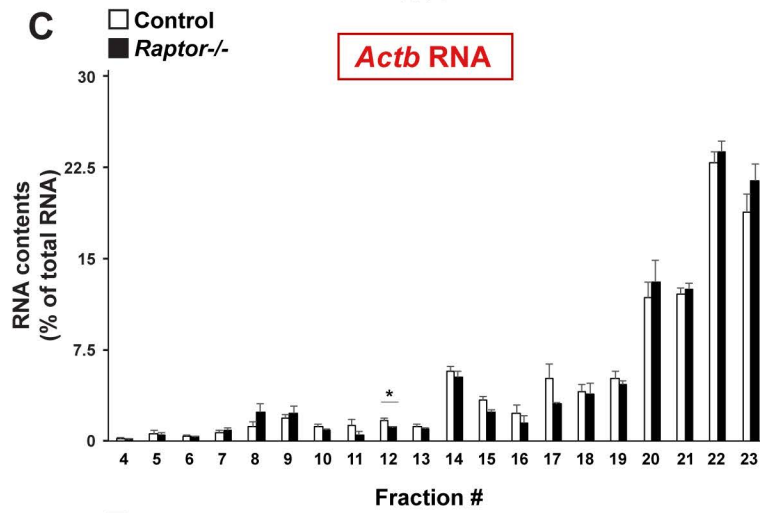
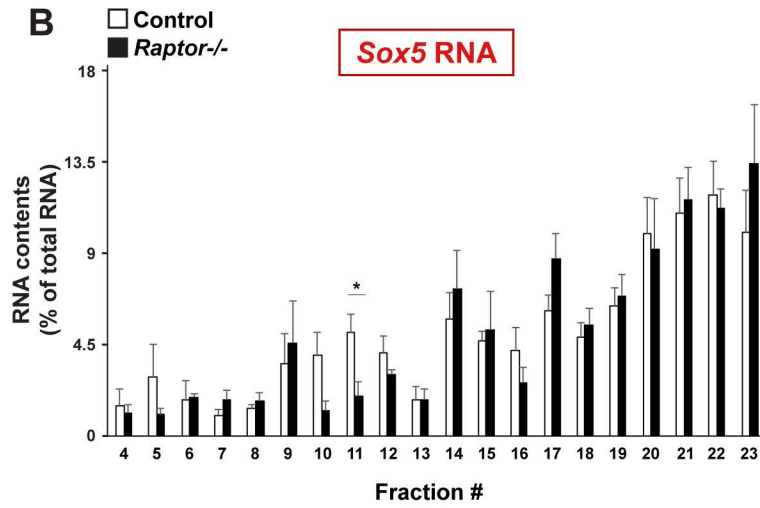
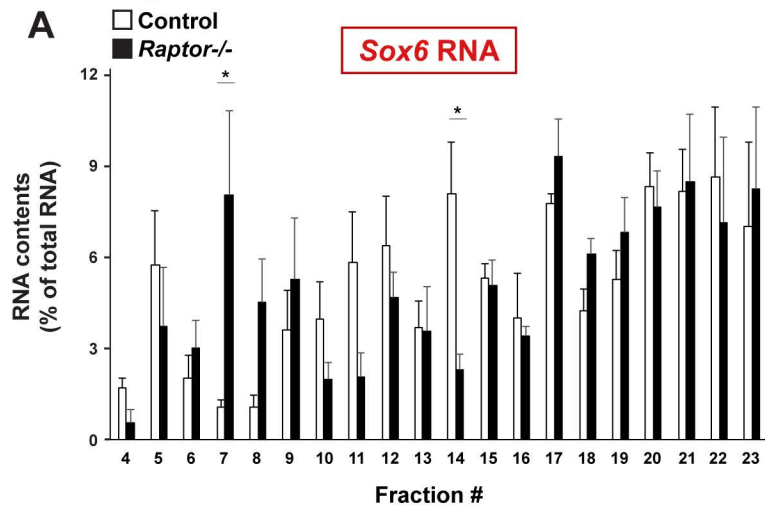
**Translational Control of Sox9 RNA by mTORC1 Contributes  
to Skeletogenesis**

**Takashi Iezaki, Tetsuhiro Horie, Kazuya Fukasawa, Makoto Kitabatake, Yuka Nakamura, Gyujin Park, Yuki Onishi, Kakeru Ozaki, Takashi Kanayama, Manami Hiraiwa, Yuka Kitaguchi, Katsuyuki Kaneda, Takayuki Manabe, Yasuhito Ishigaki, Mutsuhito Ohno, and Eiichi Hinoi**

# Supplemental Fig. 1



# Supplemental Fig. 2



## Supplemental figure legend

**Supplemental Figure 1. Histological analyses, related to Figure 1 and Figure 2.** (A) von Kossa stain of the tibia of *Prx1-Cre;Raptor<sup>fl/fl</sup>* and *Col2a1-Cre;Raptor<sup>fl/fl</sup>* embryos at E18.5. Bar=500  $\mu$ m. (B) Immunohistochemical analyses of growth plates of *Prx1-Cre;Raptor<sup>fl/fl</sup>* and *Col2a1-Cre;Raptor<sup>fl/fl</sup>* embryos at E18.5 with anti-PCNA antibody. Bar=100  $\mu$ m. N.S., not significant. (C) Whole mount immunohistochemical analyses of limb buds of *Prx1-Cre;Raptor<sup>fl/fl</sup>* embryos at E11.5. Bar=100  $\mu$ m.

**Supplemental Figure 2. Determination of RNA distribution, related to Figure 4.** Polysomes from WT cells and *Raptor*-deficient cells were sedimented in sucrose density gradients to separate efficiently 40S, 60S, 80S and polysomes, followed by determination of distribution of RNA contents (n=3 independent experiments). \*P < 0.05, \*\*P < 0.01, significantly different from the value obtained in control cells. Statistical significance was determined using the two-tailed, unpaired Student's *t*-test.

**Supplemental Table 1. List of oligonucleotides used for generation of shRNA, related to Figure 3.**

Gene	Up (5'-3')	Down (5'-3')
<i>sh4EBP1</i>	GATCCCCGGAGGCGGTGAAGA GTCACAATTCTCGAGAATTGTG ACTCTTCACCGCCTTTTTTG	AATTCAAAAAAGGCGGTGAAGA GTCACAATTCTCGAGAATTGTGA CTCTTCACCGCCTCCGGG
<i>sh4EBP2</i>	GATCCCCGTCCTGGCGCCTTAA TTGAAGACTTTCAAGAGAAGT CTTCAATTAAGGCGCCAGGATT TTTTGGAAAG	AATTCCTTCCAAAAAATCCTGGC GCCTTAATTGAAGACTTCTCTTG AAAGTCTTCAATTAAGGCGCCAG GACGGG

**Supplemental Table 2. List of primers used for real-time PCR, related to Figure 2.**

Genes	Up (5'-3')	Down (5'-3')
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<i>Runx2</i>	CCTAGTTAGAGTGGTAGCAGAAG C	ACAGACAACGAAGAAAGTTCCC AC
<i>Sox5</i>	ATTGTGCAGTCCCACAGGTTG	CTGCCTTTAGTGGGCCAGTG
<i>Sox6</i>	CGTGCCGTCTTTGTCTATCCTGG	ACCCAAGGATGGCGTGTCTAAC
<i>Sox9</i>	TTTGGGTCTGCCTGGACTGTATG G	AAGGTCTGTCCGATGTCTCTCTG C

**Supplemental Table 3. List of primers used for generation of luciferase vector, related to Figure 4.**

Genes	Up (5'-3')	Down (5'-3')
<b>5'UTR-Runx2-luc</b>	GTGAGCCAGCCCGATATTGC TTCT	ATGCTAGCCACAACAGCCACAA GTTAGC
<b>5'UTR-Sox6-luc</b>	CTCGAGAGCTGCTTTCGGCT TTC	GAAGATCTTCTTAGTTATACACG AGGATCA
<b>5'UTR-Sox9-luc</b>	CTCCGAGGAGCTCCGCTCCG ACTCGCCT	CGGAGCTCCTCGGAGGGAAAA CAGAGAAC
<b>5'UTR-Sox9<sup>TOPM</sup>-luc1</b>	GTTTCGTTCTCTGTTTTTCGGA GGTCCCTCC	AGTCGGAGCGGAGGAGGACCC TCCGAAAAC
<b>5'UTR-Sox9<sup>TOPM</sup>-luc2</b>	GGAGGGAGGAGGTCCGCTC CGACTCGCCTT	TTCTCTGTTTTTCGGAGGGAGGA GGTCCGCT

**Supplemental Table 4. List of primers used for determination of RNA content in polysome fraction, related to Figure 4.**

Genes	Up (5'-3')	Down (5'-3')
<i>Actb</i>	AAACTGGAACGGTGAAGGCGAC	CAGAAGCAATGCTGTCACCTTCC
<i>Eef2</i>	AGATCCGTGCCATCATGGA	TGGGCGATGACTGACATGTT
<i>Runx2</i>	CCTAGTTAGAGTGGTAGCAGAA GC	ACAGACAACGAAGAAAGTTCCCA C
<i>Sox5</i>	ATTGTGCAGTCCCACAGGTTG	CTGCCTTTAGTGGGCCAGTG
<i>Sox6</i>	CGTGCCGTCTTTGTCTATCCTGG	ACCCAAGGATGGCGTGTCTAAC
<i>Sox9</i>	TTTGGGTCTGCCTGGACTGTATG TG	AAGGTCTGTCCGATGTCTCTCTGC